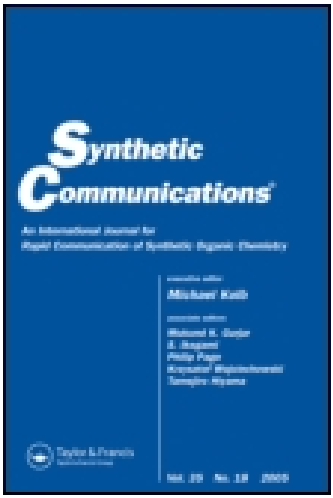

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Asymmetric Synthesis of ω-Bromo-2(S)-Methyl Acids as Precursors for Novel Arginine, Lysine, and Mercapto Residues

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Asymmetric Synthesis of ω-Bromo-2(S)-Methyl Acids as Precursors for Novel Arginine, Lysine, and Mercapto Residues

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Abstract: A series of ω-bromo-2(S)-methyl acids has been synthesized as precursors of novel arginine (Arg), lysine (Lys), and mercapto analogues. These intermediates contain α-methyl groups and are designed to mask the N-terminal amine when incorporated in pharmaceutically relevant peptides.

Keywords: Peptidomimetic, arginine, lysine, aminopeptidase, non-natural amino acid, rational drug design

INTRODUCTION

A surge of interest in the design and synthesis of small peptides and peptidomimetics as pharmaceutically relevant compounds has occurred in recent years. One strategy, designed to improve the pharmaceutical properties of native peptides, is manipulation of the N-terminal amine to mask the peptide from aminopeptidase degradation. Notably, Nα-methyl,[1]

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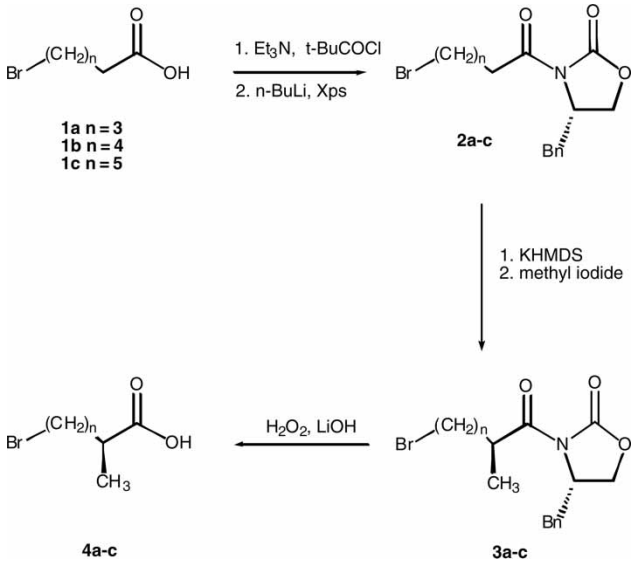
Nα-acetyl,[2] and the azido group[3] have all been used as N-terminal protecting groups. Previous work in our laboratory has defined a route for the asymmetric synthesis of ω-bromo-2(S)-azido acids as precursors for nonnatural analogues of arginine (Arg), lysine (Lys),[4] and mercapto derivatives.[5] This route utilizes the azido moiety as a protected form of the α-amine that can undergo catalytic hydrogenation at the end of the synthesis to produce the enantiomerically pure Arg, Lys or, mercapto analogues.

We also demonstrated that incorporation of the 2(S)-azido acids for the N-terminal amino acid in peptides not only improved the peptides' blood stability, but also increased their lipophilicity through removal of the charged N-terminal amine. In particular, substitution for Arg^8 in the C-terminal fragment of neurotensin, neurotensin (8–13) yielded peptide analogues with potential as novel antipsychotic compounds.[3] For further evaluation, a large-scale synthetic route is necessary to produce quantities sufficient for preclinical and clinical trials. However, large-scale synthesis of compounds containing azido groups is avoided whenever possible because of potentially explosive side-products.[6] It was hypothesized that replacement of the azido functionality with a methyl group would maintain the steric and lipophilic character associated with the azide while utilizing a synthetic route involving an inert moiety.

Starting materials **1a–c** were derivatized at the carboxylic acid with Evans' chiral auxiliary (S)-(-)-4-benzyl-2-oxazolidinone (Xps) as previously described[4,7] (Scheme 1). Addition of potassium bis(trimethylsilyl) amide (KHMDS) (5 eq.) to **2a–c** at -78°C under N_2 effected enolate formation in situ. After 30 min, methyl iodide (2 eq.) was added via cannulation and the solution was stirred at -78°C for 1 h to give **3a–c**. Removal of the chiral auxiliary with H_2O_2 and LiOH gave the respective ω-bromo-2(S)-methyl acids (**4a–c**).

Of note, quenching the methylation reaction after 2 min as previously described[7] produced poor yields of **3a–c**. The rapid quenching employed with trisyl azide is warranted because it is highly reactive, thus electrophilic addition at the α-carbon procedes at a rapid rate. However, an appreciable yield of **3a–c** was achieved only when the reaction was allowed to proceed for 1 h after methyl iodide addition. In addition, we demonstrated previously that enolization of **2b** results in immediate displacement of the ω-bromo group to form the five-membered cyclopentane carboximide.[4] To compete with the intramolecular cyclization reaction, 5 eq. of methyl iodide was added directly following the KHMDS addition. This modified procedure still gave the cyclopentane as the major product. This may be due to the less reactive nature of the methyl iodide. **3b** was separated routinely from the cyclized by-product on silica gel eluted with 100% dichloromethane. Overall, **3a–c** were produced in 95% diastereomeric excess as determined by ^{1}H NMR. The ω-bromo group of **4a–c** is easily displaced with excess nucleophile to produce a wide range of Lys analogues. In addition, this route can be applied to our synthesis of Arg analogues to create a full library of nonnatural amino acids containing a methyl group at the α-carbon.



Scheme 1. Synthesis of ω-bromo-2(S)-methyl acids.

EXPERIMENTAL FOR "C" SERIES

To a round-bottom flask containing 5 g (23.9 mmol) of **1c** was added 75 ml of anhydrous tetrahydrofuran (THF). This flask was purged with a continuous stream of argon gas and cooled to −78°C. To this flask was added 413 ml (29.6 mmol) of triethylamine (TEA) followed by dropwise addition of trimethylacetyl chloride (3.17 ml; 25.8 mmol). The flask was subsequently stirred at 0°C for 1 h. In a separate flask, 5.08 g (28.7 mmol) of (S)-(-)-4-benyl-2-oxazolidinone (Xps) was dissolved in 30 ml of anhydrous THF, cooled to −78°C, and purged with a continuous stream of argon gas. To this flask was added 11.2 ml of n-butyllithium (2.5 M solution in hexanes) and allowed to stir for 5 min. At this time, the flask containing the activated bromoacid was cooled again to −78° C and the activated Xps solution was transferred to this flask via cannula and allowed to stir for 1 h. The reaction vessel was then warmed to room temperature and the solvent removed in vacuo. The resulting residue was partitioned between 75 ml dichloromethane (DCM) and 75 ml of 0.1 M phosphate buffer (pH = 7.5). The organic fraction was saved and the phosphate buffer was extracted with additional DCM (75 ml × 2). The organic fractions were pooled, dried with $MgSO_4$, filtered, and evaporated in vacuo. The resulting oil was dissolved in ethyl acetate (EtOAc) and purified via silica-gel column chromatography eluted with 3:1 hexane–EtOAc to produce 8 g of **2c** as clear oil (91% yield).

To a round-bottom flask containing 100 ml of THF cooled to −78°C and purged with a continuous stream of argon gas was added KHMDS (24.1 ml;

12 mmol). In a separate flask purged with a continuous stream of argon gas was added 3.42 g (9.3 mmol) of **2c** dissolved in 20 ml of THF and cooled to −78°C. The solution containing **2c** was transferred via cannula to the KHMDS solution and stirred for 30 min at which time 1.16 ml (18.6 mmol) of methyl iodide was added dropwise and allowed to stir an additional 1 h. At this time, 2.3 ml of acetic acid was added to quench the reaction and the mixture was allowed to warm to room temperature over 2 h at which time the solvent was removed in vacuo. The resulting residue was partitioned between 75 ml of DCM and 75 ml of half-saturated brine. The organic fraction was saved and the half-saturated brine was extracted with additional DCM (75 ml × 2). The organic fractions were pooled, dried with $MgSO_4$, filtered, and evaporated in vacuo. The resulting oil was dissolved in EtOAc and purified via silica-gel column chromatography eluted with 3:1 hexane–EtOAc to produce 1.9 g of **3c** as clear oil (53% yield).

To 7 ml of THF and 3 ml of dH_20 in a round-bottom flask was added 0.33 g (0.86 mmol) of **3c** and cooled to 0°C. To this was added 355 μl of hydrogen peroxide (30 wt. %), followed by 71 mg (1.7 mmol) of lithium hydroxide, and the mixture was allowed to stir for 50 min at which time the reaction was quenched with 3 ml of Na_2SO_3 (0.55 g in 3 ml of H_2O) followed by 8.5 ml of 0.5 N $NaHCO_3$. The reaction was stirred for an additional 5 min at which time the solvent was removed in vacuo. The resulting residue was partitioned between 5 ml of DCM and 5 ml of dH_20. The aqueous layer was saved and additionally washed with DCM (5 ml × 2). The aqueous layer was acidified to pH = 2 using 4 N HCl and extracted with EtOAc (5 ml × 3). The organic fractions were pooled, dried with $MgSO_4$, filtered, and evaporated in vacuo to produce 167 mg of **4c** (87% yield). Purity of all compounds was assessed by ^{1}H NMR and compounds **4a–c** were subsequently incorporated into a series of neurotensin (8–13) analogues as described in a subsequent manuscript.[8]

To summarize, a method for the asymmetric synthesis of novel ω-bromo-2(S)-methyl acids is described. The α-methyl group constitutes a new strategy for protecting peptides from aminopeptidase degradation. Notably, N-terminal methyl analogues of neurotensin (8–13) retained the receptor-binding affinity of the parent peptide while demonstrating significant increases in CNS activity in animal models when administered peripherally or orally.[8]

ANALYTICAL DATA

[3(2S),4S]-3-(2-Methyl-5-bromo-1-oxovaleryl)-4-(phenylmethyl)-2-oxazolidinone (3a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.15 (m, 5H), 4.71–4.63 (m, 1H), 4.18–4.15 (d, J = 5.0 Hz, 2H), 3.71–3.65 (m, 1H), 3.41–3.33 (m, 2H), 3.27–3.20 (dd, J = 4.0, 13.8 Hz, 1H), 2.89–2.81 (dd, J = 10.0, 14.2 Hz, 1H), 1.90–1.55 (m, 4H), 1.24–1.20 (d, J = 7.4, 3H); ^{13}C NMR

(100 MHz, $CDCl_3$) δ 176.8, 153.2, 135.2, 129.6, 129.1, 127.6, 66.4, 55.6, 38.3, 37.6, 33.8, 32.2, 31.8, 17.9.

[3(2S),4S]-3-(2-Methyl-6-bromo-1-hexanoyl)-4-(phenylmethyl)-2-oxazolidinone (3b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.19 (m, 5H), 4.72–4.65 (m, 1H), 4.25–4.16 (d $J = 4.2$ Hz, 2H), 3.77–3.67 (m, 1H), 3.46–3.36 (t, $J = 7.0$ Hz, 2H), 3.29–3.22 (dd, $J = 4.0$, 14.0 Hz, 1H), 2.82–2.74 (dd, $J = 9.0$, 14.0 Hz, 1H), 1.92–1.74 (m, 3H), 1.50–1.42 (m, 3H), 1.25–1.21 (d, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 153.2, 135.3, 129.6, 129.1, 66.4, 55.6, 38.1, 37.8, 34.1, 32.8, 32.5, 26.1, 18.7.

[3(2S),4S]-3-(2-Methyl-7-bromo-1-oxoheptyl)-4-(phenylmethyl)-2-oxazolidinone (3c): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.22 (m, 5H), 4.74–4.66 (m, 1H), 4.25–4.19 (d, $J = 4.0$ Hz, 2H), 3.77–3.70 (m, 1H), 3.45–3.39 (t, $J = 7.0$ Hz, 2H), 3.31–3.23 (dd, $J = 3.7$, 13.7 Hz, 1H), 2.84–2.77 (dd, $J = 10.0$, 12.5 Hz, 1H), 1.91–1.77 (m, 3H), 1.50–1.32 (m, 5H), 1.25–1.20 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.2, 153.1, 135.4, 129.7, 129.1, 127.5, 66.4, 55.7, 38.2, 37.9, 34.3, 33.4, 32.8, 28.4, 27.7, 17.8.

2(S)-Methyl-5-bromovaleric Acid (4a): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.46–3.38 (t, $J = 6.0$ Hz, 2H), 2.56–2.46 (m, 1H), 1.95–1.60 (m, 4H), 1.25–1.20 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1, 38.9, 33.6, 32.1, 30.5, 17.3.

2(S)-Methyl-6-bromohexanoic Acid (4b): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.45–3.38 (t, $J = 6.2$ Hz, 2H), 2.55–2.45 (m, 1H), 1.92–1.85 (m, 2H), 1.77–1.68 (m, 1H), 1.55–1.46 (m, 3H), 1.24–1.19 (d, $J = 7.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.5, 39.5, 33.8, 32.9, 32.7, 26.0, 17.2.

2(S)-Methyl-7-bromoheptanoic Acid (4c): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.43–3.36 (t, $J = 6.8$ Hz, 2H), 2.51–2.42 (m, 1H), 1.90–1.64 (m, 3H), 1.49–1.32 (m, 5H) 1.20–1.14 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.6, 39.5, 34.1, 33.5, 32.7, 28.2, 26.6, 17.1.

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